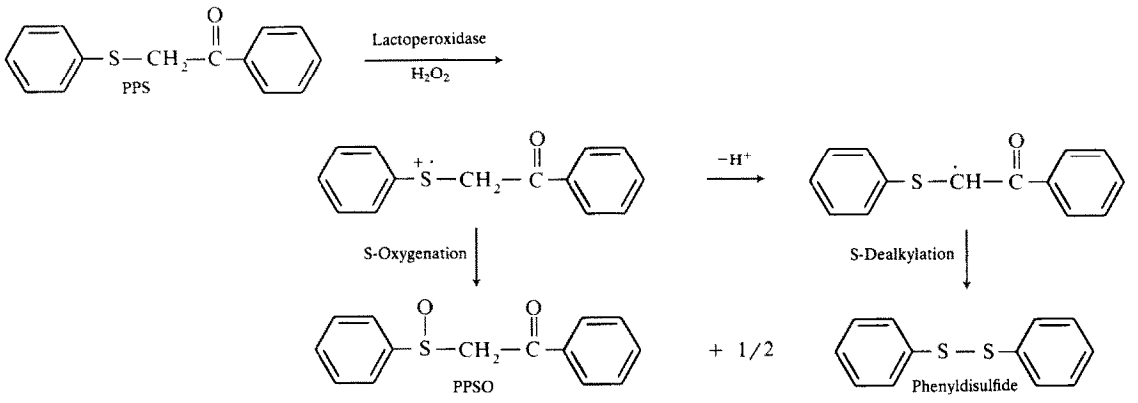

Scheme 2. S-Oxygenation and S-dealkylation reactions of PPS.

presence of a one-electron S-oxidized intermediate was inferred from the use of an organosulfide substrate containing acidic alpha-protons. These results provide support for a common S-oxygenation mechanism for LPX substrates and inactivators and are consistent with the formation of reactive S-oxygenated products by the action of LPX on thiocarbamide goitrogens [3].

*Departments of *Agricultural Biochemistry and ‡Chemistry*
University of Hawaii
Honolulu, HI 96822,
U.S.A.

DANIEL R. DOERGE*†
GERALD L. PITZ‡
DOUGLAS P. ROOT‡

† Send correspondence to: Dr. Daniel R. Doerge, Department of Agricultural Biochemistry, University of Hawaii at Manoa, 1800 East West Rd., Honolulu, HI 96822.

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Effect of histamine on $^{45}Ca^{2+}$ uptake in rat brain synaptosomes

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Ca^{2+} is regarded as an intracellular messenger involved in coupling stimulus to response in a wide variety of biological reactions [1]. Among other functions, it is well established that calcium ions play a central role in the stimulus–neurosecretion coupling [2, 3]. It has been shown that Ca^{2+} is accumulated by nerve terminals during depolarization and this Ca^{2+} entry subsequently results in neurotransmitter (NT) release [4, 5]. Although the regulatory mechanisms involved in the neurosecretory process are still largely unknown, evidence has been provided that at least certain NT or neuromodulators exert their control on this process by regulating Ca^{2+} fluxes into nerve terminals, thereby increasing or restricting the availability of Ca^{2+} necessary for the release process [5–8].

Accumulating evidence strongly suggests that histamine (HA) acts as a NT in the CNS [9]. HA has been shown to either inhibit its own release [10] or to stimulate that of catecholamines [11, 12] and serotonin [13] in the mammalian brain. The mechanism by which HA regulates NT release has yet to be elucidated. Indirect evidence indicates, however, that it is a Ca^{2+}-dependent process [10, 12]. With this insight, we studied, as a preliminary approach, the effect of HA on the uptake of $^{45}Ca^{2+}$ into rat brain synaptosomes.

Materials and methods

Materials. $^{45}CaCl_2$ (10–40 mCi/mg calcium) was purchased from Amersham International. Histamine, mepyr-

amine and diphenhidramine were obtained from Sigma Chemical (F.R.G.). Impromidine, 2-tiazoliletilamine and dimaprit were kindly supplied by Smith Kline & French. Ranitidine was a gift from Lesvi Laboratories (Spain), $LaCl_3$ was obtained from Fluka Ag. (Switzerland). All other reagents were of analytical grade.

Preparation of synaptosomes. Synaptosomes were prepared from whole brains of female Sprague–Dawley rats, weighing 150–200 g, using the method of Hajos [14]. The final synaptosomal pellet was suspended in an appropriate volume of buffered 0.32 M sucrose solution, pH 7.4 to give a protein concentration of 5–10 mg/ml.

Protein was determined by the method of Lowry *et al.* [15] using bovine serum albumin as a standard.

$^{45}Ca^{2+}$ *uptake.* $^{45}Ca^{2+}$ uptake to synaptosomes was carried out by the filtration method described by Wu *et al.* [7] with some modifications. Briefly, aliquots of the synaptosome suspension (containing 0.25–0.50 mg protein/ml) were preincubated for a period of 20 min at 37° in Ringer–Tris medium (R–T) containing (final concentrations, mM): NaCl 130; KCl 5; $MgCl_2$ 1.2; $CaCl_2$ 1.2; glucose 10; Tris 20 adjusted to pH 8 with HCl and readjusted to pH 7.4 with a 95% O_2–5% CO_2 atmosphere. Histamine and histaminergic drugs were added 10 min prior to the addition of $^{45}Ca^{2+}$. (Preliminary experiments showed that HA-induced uptake of $^{45}Ca^{2+}$ did not occur immediately; a period of 1 min was needed to observe a response to the amine (data not shown).

$^{45}Ca^{2+}$ uptake into synaptosomes was initiated by the addition of 0.3 μCi/ml of $^{45}CaCl_2$ and allowed to proceed at fixed or different times depending on the experiment (see Results). Incubations were terminated by a 10-fold dilution of the samples with ice-cold R–T medium with an additional 5 mM La^{3+} followed by rapid filtration through GF/C Whatman glass fiber filters prewashed with 10 ml of La^{3+}–Tris buffer (5 mM $LaCl_3$, 10 mM Tris–HCl, pH 7.4) on a Millipore vacuum filtration manifold. After three separate 5 ml washes with ice-cold R–T–La^{3+} medium, filters were placed in scintillation vials and dried in an oven at 100°. Dried filters were counted for radioactivity after the addition of 3 ml of Unisolve (Koch and Light) on a Beckmann LS800 scintillation spectrometer.

Results are expressed as nmole Ca^{2+}/mg protein and represent the net uptake of Ca^{2+} (total uptake minus blank value). The blank value represents the sum of nonspecific absorption of $^{45}Ca^{2+}$ to synaptosomes, to contaminating particles and to the filters. It was estimated by determining the radioactivity of parallel samples diluted 10-fold prior to the addition of $^{45}Ca^{2+}$, following thereafter the same procedure as described above.

Statistics. The data are presented as means ± SEM. The mean values were compared by variance analysis. In some cases, when F was significant, the difference between means was determined using the method of Scheffe's.

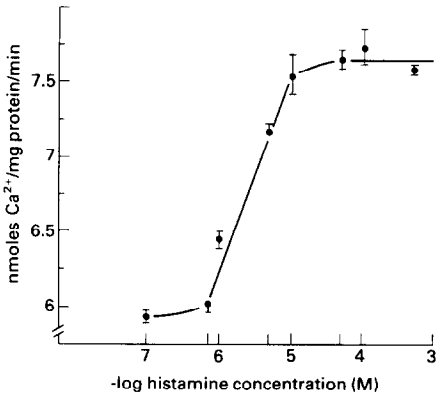

Fig. 1. Effect of HA on $^{45}Ca^{2+}$ uptake by rat brain synaptosomes as a function of HA concentration. $^{45}Ca^{2+}$ uptake by synaptosomes was measured at 1 min in the presence of various concentrations of HA (10^{-7}–5×10^{-4} M) in R–T medium as described in Methods. The control value for Ca^{2+} uptake was 5.793 ± 0.0509 nmoles Ca^{2+}/mg protein (N = 5). Values are means ± SEM, N = 5. Statistical significant differences became apparent at 10^{-6} M HA with a $P < 0.001$ (Scheffe's test).

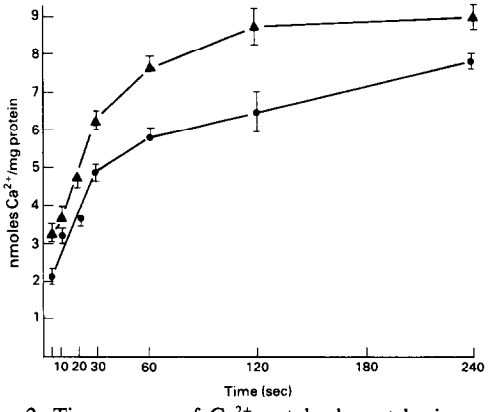

Fig. 2. Time course of Ca^{2+} uptake by rat brain synaptosomes. Synaptosomes (0.25–0.5 mg protein/ml) were preincubated for 20 min at 37° in R–T medium containing 1.2 mM $CaCl_2$. Uptake was initiated by the addition of $^{45}CaCl_2$ (specific activity 0.243 μCi/μmol Ca^{2+}) and terminated at the indicated times by a 10-fold dilution with stopping solution and rapidly filtering as described in methods. Ca^{2+} taken up by synaptosomes in the absence (●), and in the presence (▲) of 10^{-4} M HA. Each point represents a mean ± SEM, with N = 6.

Results and discussion

The ability of HA to induce an increase in the uptake of $^{45}Ca^{2+}$ into synaptosomes was assessed by examining the effect produced by various concentrations of HA incubated for 60 sec with $^{45}Ca^{2+}$. A concentration–effect relationship for the increase in $^{45}Ca^{2+}$ uptake in response to HA is presented in Fig. 1. Maximal stimulation of $^{45}Ca^{2+}$ uptake was elicited at 5×10^{-5} M HA and was of 33% over control uptake levels. Half-maximal response occurred at about 4×10^{-6} M HA.

The results of the time course of $^{45}Ca^{2+}$ uptake into synaptosomes in response to HA are shown in Fig. 2. Measurement of $^{45}Ca^{2+}$ uptake was done at 5, 10, 15, 30, 120 and 240 sec in both the absence and presence of 10^{-4} M HA. At all times, HA increased $^{45}Ca^{2+}$ uptake. The maximal increase (35% over control levels) was reached at 60 sec. Two-way-analysis of variance of data in Fig. 2. indicated that the increase in the $^{45}Ca^{2+}$ uptake into synaptosomes was both dependent on time ($P < 0.001$) and HA ($P < 0.01$). No statistically significant interactions of time × HA were observed. The time course of HA-induced $^{45}Ca^{2+}$ uptake into synaptosomes paralleled that of controls. The rate of HA-induced $^{45}Ca^{2+}$ uptake was maximal during the initial 30 sec, progressively declined thereafter and reached a steady-state level after 60 sec. In an attempt to identify the HA receptor mediating the increased uptake of Ca^{2+} into synaptosomes in response to HA, several histaminergic drugs were tested. The results of the pharmacological study are presented in Fig. 3. HA-induced $^{45}Ca^{2+}$ uptake was measured at 1 min. The increase of uptake of $^{45}Ca^{2+}$ induced by 10^{-4} M HA was of 7.479 ± 0.048 nmole Ca^{2+}/mg protein (35% over control levels). H_2 receptor antagonists, cimetidine and ranitidine at concentrations of 10^{-5} M and 10^{-6} M, respectively, com-

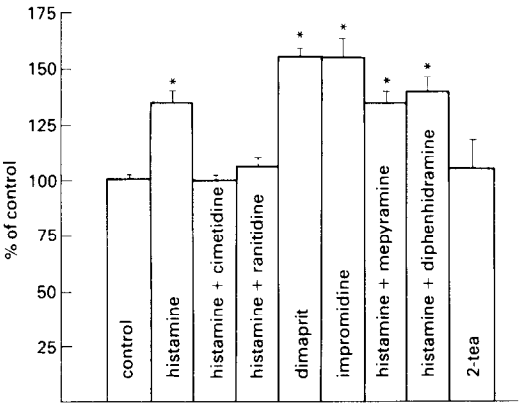

Fig. 3. Effect of HA antagonists and agonists on $^{45}Ca^{2+}$ uptake by rat brain synaptosomes. Uptake was measured at 1 min in R–T medium as described in Methods. Histamine 10^{-4} M: cimetidine 10^{-5} M; ranitidine 10^{-6} M; mepyramine and diphenhidramine 10^{-7} M; impromidine 5×10^{-5} M; dimaprit 10^{-4} M; 2-thiazoliletilamine (2-tea) 10^{-4} M. Data are expressed as percentage of controls. The control value for Ca^{2+} uptake was 5.526 ± 0.05. Results are means ± SEM; N = 5. (*) $P < 0.001$ vs control; Scheffe's test.

pletely blocked the effect of 10^{-4} M HA. In contrast, H_1 receptor antagonists, mepyramine and diphenhidramine, at 10^{-7} M did not alter the response induced by HA. In no case did either H_1 or H_2 receptor antagonists alone affect control $^{45}Ca^{2+}$ uptake levels (data not shown).

Dimaprit and Impromidine, selective H_2 receptor agonists at 10^{-4} M and 5×10^{-5} M, respectively, mimicked the effect of HA, whereas the H_1 receptor agonist 2-thiazoliletialmine (2-TEA) at 10^{-4} M was ineffective in modifying $^{45}Ca^{2+}$ uptake into synaptosomes with respect to controls. These results strongly suggest that HA-induced uptake of calcium into synaptosomes is mediated by H_2 receptors.

As already stated in the Introduction, the necessity of calcium ions for the process of NT release is well documented. Consistent with this generalized assumption, it has been recently reported that HA-induced release of dopamine from rabbit caudate nucleus slices as well as of acetylcholine in the guinea-pig ileum both depend critically on the extracellular Ca^{2+} concentration [12, 16]. Moreover, according to Pilc *et al.* [13], HA influence on the serotonergic system is mediated by HA H_2 receptors. The same receptor has been reported to be involved in the HA-induced release of dopamine in mammalian brain slices [12].

As both the release of the mentioned NT and the uptake of Ca^{2+} in response to HA seem to be mediated by H_2 receptors, it is plausible that these processes are related in some way. Therefore, we suggest that HA-induced entry of Ca^{2+} into nerve terminals could constitute an essential step for triggering the release of catecholamines or serotonin. Work is in progress to test this hypothesis.

In summary, our preliminary data indicate that HA induces an increased uptake of $^{45}Ca^{2+}$ into rat brain synaptosomes in a dose-dependent manner. This effect seems to be mediated by H_2 receptors. These findings could be related to a mechanism of control of HA-dependent NT release.

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Departamento de Bioquimica y Biologia Molecular
Facultad de Medicina
Universidad Autonóma de Barcelona
08193 Bellaterra
Barcelona, Spain

RICARDO RODRIGUEZ*
REGINA BRANDNER
JOSEFA SABRIÁ
ALFONSO TOLEDO
JOSÉ RODRIGUEZ
ISAAC BLANCO

* To whom correspondence should be sent.

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Cyclic AMP efflux from rat striatal slices is enhanced by CCK

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Cholecystokinin (CCK) is one of several peptides which have been localised to neuronal elements of the basal ganglia of various species [1] and histological evidence has demonstrated that CCK and dopamine (DA) are co-localised in a large proportion of neurons in the mesolimbic system and medial substantia nigra [2–4], raising the possibility that CCK may modulate dopaminergic function.

CCK and dopamine have been shown to interact in a